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## CLAIMS

What is claimed is:

1. A cell culture container comprising:  
a supporting container comprising a first side wall connected to a portion of an opposing second side wall along a peripheral seal to define a containment area, each side wall having an interior surface, the first side wall being constructed from a gas permeable material selected from the group consisting of: polymeric material, paper, and fabric, the first side wall having a gas permeability sufficient to permit cellular respiration, and the second side wall being constructed from a material selected from the group consisting of: polymeric material, paper, fabric, and foil; and  
a fibrin matrix layer on a portion of the interior surface of the first side wall or the second side wall of the supporting container.
2. The cell culture container of claim 1 wherein the gas permeable material is selected from the group consisting of: ethylene vinyl acetate copolymers, polyolefins, polyamides, polyesters, styrene and hydrocarbon copolymers, and fluorocarbon elastomers.
3. The cell culture container of claim 1 wherein the polymeric material of the first side wall or the second side wall of the supporting container is a multiple-component polymer blend.
4. The cell culture container of claim 3 wherein at least one of the components of the multiple-component polymer blend is a styrene and hydrocarbon copolymer.
5. The cell culture container of claim 1 wherein the gas permeable material is a monolayer structure.
6. The cell culture container of claim 1 wherein the gas permeable material is a multilayer structure.
7. The cell culture container of claim 6 wherein the multilayer structure comprises:  
a first layer comprising a first ethylene vinyl acetate copolymer, the first layer having a first surface and a second surface; and

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a second layer adhering to the first surface of the first layer, the second layer comprising a second ethylene vinyl acetate copolymer;

wherein the second surface of the first layer forms the interior surface of the supporting container.

8. The cell culture container of claim 7 wherein the first ethylene vinyl acetate copolymer having a vinyl acetate content of greater than 18% by weight of the copolymer.

9. The cell culture container of claim 7 wherein the second ethylene vinyl acetate copolymer having a vinyl acetate content of less than 18% by weight of the copolymer.

10. The cell culture container of claim 7 wherein the first ethylene vinyl acetate copolymer having a vinyl acetate content of about 18% by weight of the copolymer.

11. The cell culture container of claim 7 wherein the second ethylene vinyl acetate copolymer having a vinyl acetate content of about 9% by weight of the copolymer.

12. The cell culture container of claim 6 wherein the multilayer structure comprises:

a first layer comprising polystyrene having a thickness within the range of 0.0001 inches to about 0.0010 inches; and

a second layer adhering to the first layer, the second layer comprising a polymeric material selected from the group consisting of ethylene vinyl acetate copolymers, polyolefins, polyamides, polyesters, styrene and hydrocarbon copolymers, fluorocarbon elastomers, the second layer having a thickness within the range of 0.004 inches to about 0.025 inches.

13. The cell culture container of claim 12, wherein the polymeric material of the second layer is a multi-component polymer blend.

14. The cell culture container of claim 13, wherein at least one of the components of the multi-component polymer blend is a styrene and hydrocarbon copolymer.

15. The cell culture container of claim 12, wherein the fibrin matrix is positioned on a portion of the polystyrene layer covering substantially an entire surface of the polystyrene layer.

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16. The cell culture container of claim 1, wherein the second side wall is constructed from a gas permeable material selected from the group consisting of: polymeric materials, paper, and fabric.

17. The cell culture container of claim 16, wherein the polymeric material of the second side wall is selected from the group consisting of: ethylene vinyl acetate copolymers, polyolefins, polyamides, polyesters, styrene and hydrocarbon copolymers, and fluorocarbon elastomers.

18. The cell culture container of claim 17, wherein at least one of the components of the multi-component polymer blend is a styrene and hydrocarbon copolymer.

19. The cell culture container of claim 16, wherein the gas permeable material is a monolayer structure.

20. The cell culture container of claim 16, wherein the gas permeable material is a multilayer structure.

21. The cell culture container of claim 20, wherein the multilayer structure comprises:

- a first layer comprising a third ethylene vinyl acetate copolymer with a vinyl acetate content of greater than 18% by weight of the copolymer, the first layer having a first surface and a second surface; and

- a second layer adhering to the first surface of the first layer, the second layer comprising a fourth ethylene vinyl acetate copolymer with a vinyl acetate content of from less than 18% by weight of the copolymer,

- wherein the second surface of the first layer forms the inner surface of the container.

22. The cell culture container of claim 21, wherein the a vinyl acetate content of the fourth vinyl acetate copolymer in the first layer is about 18% by weight of the copolymer.

23. The cell culture container of claim 21 wherein the a vinyl acetate content of the fifth vinyl acetate copolymer in the second layer is about 9% by weight of the copolymer.

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24. The cell culture container of claim 20, wherein the multilayer structure comprises:

a first layer comprising polystyrene having a thickness within the range of 0.0001 inches to about 0.0010 inches; and

a second layer adhering to the first layer, the second layer comprising a polymeric material selected from the group consisting of ethylene vinyl acetate copolymers, polyolefins, polyamides, polyesters, styrene and hydrocarbon copolymers, fluorocarbon elastomers, the second layer having a thickness within the range of 0.004 inches to about 0.025 inches.

25. The cell culture container of claim 24, wherein the polymeric material of the second layer is a multi-component polymer blend.

26. The cell culture container of claim 25, wherein at least one of the components of the multi-component polymer blend is a styrene and hydrocarbon copolymer.

27. The cell culture container of claim 1, wherein the container having an oxygen permeability of from about 9 to about 15 Barrers, a carbon dioxide permeability of from about 40 to about 80 Barrers, a nitrogen permeability of from about 10 to about 100 Barrers, and a water vapor transmission rate of less than about 20 (g mil/100 in<sup>2</sup>/day).

28. The cell culture container of claim 1, wherein the first side wall and the second side wall having a flexural modulus of from about 10,000 to about 30,000 psi as measured according to ASTM D-790.

29. The cell culture container of claim 1, wherein at least a portion of the container is optically clear.

30. The cell culture container of claim 1, wherein a substantial portion of the container is optically clear.

31. The cell culture container of claim 1, wherein the container is radiation sterilizable.

32. The cell culture container of claim 1, wherein the container further comprising at least one port providing access to the containment area.

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33. The cell culture container of claim 1, wherein the fibrin matrix extends over substantially an entire surface of the interior surface of at least one of the side walls.

34. The cell culture container of claim 1, wherein the fibrin matrix is on at least a portion of the interior surface of each of the side walls.

35. The cell culture container of claim 1, wherein the fibrin matrix is three-dimensional with pore sizes of from about 0.5 to about 5.0  $\mu\text{m}$  in diameter.

36. The cell culture container of claim 1, wherein the fibrin matrix is formed by cross-linking fibrin or fibrinogen.

37. The cell culture container of claim 1, wherein the fibrin matrix is prepared by mixing a first solution comprising fibrinogen and factor XIII with a second solution comprising thrombin and calcium to form the fibrin matrix.

38. The cell culture container of claim 37, wherein the fibrinogen is derived from mammalian plasma.

39. The cell culture container of claim 38, wherein the mammalian plasma is human plasma.

40. The cell culture container of claim 37, wherein the fibrinogen is a recombinant fibrinogen.

41. The cell culture container of claim 37, wherein the factor XIII is derived from mammalian plasma.

42. The cell culture container of claim 41, wherein the mammalian plasma is human plasma.

43. The cell culture container of claim 37, wherein the factor XIII is a recombinant factor XIII.

44. The cell culture container of claim 37, wherein the thrombin is derived from mammalian plasma.

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45. The cell culture container of claim 44, wherein the mammalian plasma is selected from the group consisting of bovine plasma and human plasma.

46. The cell culture container of claim 37, wherein the thrombin is a recombinant thrombin.

47. The cell culture container of claim 37 wherein the concentration of fibrinogen in the first solution is from about 2.0 to about 20 mg/mL, the concentration of the factor XIII in the first solution is from about 10 to about 40 IU/mL, the concentration of the thrombin in the second solution is from about 2.5 IU/mL to about 50 IU/mL, and the concentration of the calcium in the second solution is from about 40 to about 100 umoles/mL. Approximately 0.5-1.0 mLs of the first solution is mixed with 0.5-1.0 mLs of the second solution to form a fibrin-forming mixture. The polymerization reaction takes place at room temperature in 1-5 minutes and is complete in about 5-15 minutes at 37°C. The fibrin matrix formed in this embodiment has a pore size of about 0.5 –5.0  $\mu\text{m}$  in diameter.

48. A cell culture container comprising:  
a supporting container comprising a first side wall connected to a portion of an opposing second side wall along a peripheral seal to define a containment area, each side wall having an interior surface, the first side wall and the second side wall are constructed from an ethylene vinyl acetate copolymer having a gas permeability sufficient to permit cellular respiration; and  
a fibrin matrix layer on a portion of the interior surface of the first side wall or the second side wall of the supporting container.

49. The cell culture container of claim 47, wherein the ethylene vinyl acetate copolymer is a multilayer structure comprising:  
a first layer comprising a fifth ethylene vinyl acetate copolymer with a vinyl acetate content of greater than 18% by weight of the copolymer, the first layer having a first surface and a second surface; and  
a second layer adhering to the first surface of the first layer, the second layer comprising a sixth ethylene vinyl acetate copolymer with a vinyl acetate content of less than 18% by weight of the copolymer;

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wherein the second surface of the first layer forms the interior surface of the supporting container.

50. The cell culture container of claim 48, wherein the vinyl acetate content of the sixth ethylene vinyl acetate copolymer is about 18% by weight of the copolymer, and the vinyl acetate content of the seventh ethylene vinyl acetate copolymer is about 9% by weight of the copolymer.

51. A cell culture container comprising:

a supporting container comprising a first side wall connected to a portion of an opposing second side wall along a peripheral seal to define a containment area, each side wall having an interior surface, the side walls are constructed from a multilayer gas permeable polymeric structure having a gas permeability sufficient to permit cellular respiration, and the multilayer polymeric structure comprising:

a first layer comprising polystyrene having a thickness within the range of 0.0001 inches to about 0.0010 inches; and

a second layer adhering to the first layer, the second layer comprising a polymeric material selected from the group consisting of ethylene vinyl acetate copolymers, polyolefins, polyamides, polystyrene and hydrocarbon copolymers, the second layer having a thickness within the range of 0.004 inches to about 0.025 inches; and

a fibrin matrix layer on a portion of the interior surface of the first side wall or the second side wall of the supporting container.

52. The cell culture container of claim 51, wherein the polymeric material of the second layer is a multi-component polymer blend.

53. The cell culture container of claim 52, wherein at least one of the components of the multi-component polymer blend is a styrene and hydrocarbon copolymer.

54. A method of culturing cells, the method comprising the steps of:

providing a flexible gas permeable container, the container comprising:

a supporting container comprising a first side wall connected to a portion of an opposing second side wall along a peripheral seal to define a containment area, each side wall having an interior surface, the first side wall being constructed from a gas permeable material selected from the group consisting of: polymeric material, paper, and fabric, the first side wall

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having a gas permeability sufficient to permit cellular respiration, and the second side wall being constructed from a material selected from the group consisting of: polymeric material, paper, fabric, and foil;

forming a fibrin matrix layer on a portion of the interior surface of at least one of the side walls of the supporting container; and

introducing a cell line into the containment area of the container to allow the cells to attach to the fibrin matrix.

55. The method of claim 54, wherein the gas permeable material is selected from the group consisting of: ethylene vinyl acetate copolymers, polyolefins, polyamides, polyesters, styrene and hydrocarbon copolymers fluorocarbon elastomers.

56. The method of claim 55, wherein the polymeric material of the first side wall or the second side wall is a multiple-component polymer blend.

57. The method of claim 56, wherein at least one of the components of the multiple-component polymer blend is a styrene and hydrocarbon copolymer.

58. The method of claim 54, wherein the gas permeable material is a monolayer structure.

59. The method of claim 54, wherein the gas permeable material is a multilayer structure.

60. The method of claim 59, wherein the multilayer structure comprises:  
a first layer comprising a seventh ethylene vinyl acetate copolymer, the first layer having a first surface and a second surface; and  
a second layer adhering to the first surface of the first layer, the second layer comprising an eighth ethylene vinyl acetate copolymer;  
wherein the second surface of the first layer forms the interior surface of the supporting container.

61. The method of claim 60, wherein the vinyl acetate content of the ninth vinyl acetate copolymer is greater than 18% by weight of the copolymer.



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62. The method of claim 60, wherein the vinyl acetate content of the tenth vinyl acetate copolymer is less than 18% by weight of the copolymer.

63. The method of claim 60, wherein the vinyl acetate content of the ninth vinyl acetate copolymer is about 18% by weight of the copolymer.

64. The method of claim 60, wherein the vinyl acetate content of the tenth vinyl acetate copolymer is about 9% by weight of the copolymer.

65. The method of claim 60, wherein the multilayer structure comprises:  
a first layer comprising polystyrene having a thickness within the range of 0.0001 inches to about 0.0010 inches; and  
a second layer adhering to the first layer, the second layer comprising a polymeric material selected from the group consisting of ethylene vinyl acetate copolymers, polyolefins, polyamides, polyesters, styrene and hydrocarbon copolymers, fluorocarbon elastomers, the second layer having a thickness within the range of 0.004 inches to about 0.025 inches.

66. The method of claim 65, wherein the polymeric material of the second layer is a multi-component polymer blend.

67. The method of claim 66, wherein at least one of the components of the multi-component polymer blend is a styrene and hydrocarbon copolymer.

68. The method of claim 65, wherein the fibrin matrix is positioned on a portion of the polystyrene layer covering substantially an entire surface of the polystyrene layer.

69. The method of claim 54, wherein the second side wall of the supporting container is constructed from a gas permeable material selected from the group consisting of: polymeric materials, paper, and fabric.

70. The method of claim 69, wherein the polymeric material of the second side wall is selected from the group consisting of: ethylene vinyl acetate copolymers, polyolefins, polyamides, polyesters, styrene and hydrocarbon copolymers, and fluorocarbon elastomers.

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71. The method of claim 70, wherein at least one of the components of the multi-component polymer blend is a styrene and hydrocarbon copolymer.

72. The method of claim 69, wherein the gas permeable material is a monolayer structure.

73. The method of claim 69, wherein the gas permeable material is a multilayer structure.

74. The method of claim 73, wherein the multilayer structure comprises:  
a first layer comprising a ninth ethylene vinyl acetate copolymer with a vinyl acetate content of greater than 18% by weight of the copolymer, the first layer having a first surface and a second surface; and  
a second layer adhering to the first surface of the first layer, the second layer comprising a tenth ethylene vinyl acetate copolymer with a vinyl acetate content of less than 18% by weight of the copolymer;  
wherein the second surface of the first layer forms the inner surface of the supporting container.

75. The method of claim 74, wherein the a vinyl acetate content of the ninth vinyl acetate copolymer in the first layer is about 18% by weight of the copolymer.

76. The method of claim 74, wherein the a vinyl acetate content of the tenth vinyl acetate copolymer in the second layer is about 9% by weight of the copolymer.

77. The method of claim 73, wherein the multilayer structure comprises:  
a first layer comprising polystyrene having a thickness within the range of 0.0001 inches to about 0.0010 inches; and  
a second layer adhering to the first layer, the second layer comprising a polymeric material selected from the group consisting of ethylene vinyl acetate copolymers, polyolefins, polyamides, polyesters, styrene and hydrocarbon copolymers, the second layer having a thickness within the range of 0.004 inches to about 0.025 inches.

78. The method of claim 77, wherein the polymeric material of the second layer is a multi-component polymer blend.

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79. The method of claim 78, wherein at least one of the components of the multi-component polymer blend is a styrene and hydrocarbon copolymer.

80. The method of claim 54, wherein the container having an oxygen permeability of from about 9 to about 15 Barrers, a carbon dioxide permeability of from about 40 to about 80 Barrers, a nitrogen permeability of from about 10 to about 100 Barrers, and a water vapor transmission rate of less than about 20 (g mil/100 in<sup>2</sup>/day).

81. The method of claim 54, wherein the first side wall and the second side wall having a flexural modulus of from about 10,000 to about 30,000 psi as measured according to ASTM D-790.

82. The method of claim 54, wherein at least a portion of the container is optically clear.

83. The method of claim 54, wherein a substantial portion of the container is optically clear.

84. The method of claim 54, wherein the container is radiation sterilizable.

85. The method of claim 54, wherein the container further comprising at least one port providing access to the containment area.

86. The method of claim 54, wherein the fibrin matrix extends over substantially an entire surface of the interior surface of at least one of the side walls.

87. The method of claim 54, wherein the fibrin matrix is on at least a portion of the interior surface of each of the side walls.

88. The method of claim 54, wherein the fibrin matrix is three-dimensional with pore sizes of from about 0.5 to about 5.0 um in diameter.

89. The method of claim 54, wherein the fibrin matrix is formed by cross-linking fibrin or fibrinogen.

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90. The method of claim 54, wherein the fibrin matrix is prepared by mixing a first solution comprising fibrinogen and factor XIII with a second solution comprising thrombin and calcium to form the fibrin matrix.

91. The method of claim 90, wherein the fibrinogen is derived from mammalian plasma.

92. The method of claim 91, wherein the mammalian plasma is human plasma.

93. The method of claim 90, wherein the fibrinogen is a recombinant fibrinogen.

94. The method of claim 90, wherein the factor XIII is derived from mammalian plasma.

95. The method of claim 94, wherein the mammalian plasma is human plasma.

96. The method of claim 90, wherein the factor XIII is a recombinant factor XIII.

97. The method of claim 90, wherein the thrombin is derived from mammalian plasma.

98. The method of claim 97, wherein the mammalian plasma is selected from the group consisting of bovine plasma and human plasma.

99. The method of claim 90, wherein the thrombin is a recombinant thrombin.

100. The method of claim 90, wherein the concentration of fibrinogen in the mixture in the first solution is from about 2.0 to about 20 mg/mL, the concentration of the factor XIII in the first solution is from about 10 to about 40 IU/mL, the concentration of the thrombin in the second solution is from about 2.5 IU/mL to about 50 IU/mL, and the concentration of the calcium in the second solution is from about 40 to about 100 umoles/mL. Approximately 0.5-1.0 mLs of the first solution is mixed with 0.5-1.0 mLs of the second solution to form a fibrin-forming mixture. The polymerization reaction takes place at room temperature in 1-5 minutes and is complete in about 5-15 minutes at 37°C. The fibrin matrix formed in this embodiment has a pore size of about 0.5 –5.0  $\mu\text{m}$  in diameter.

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101. The method of claim 54, further comprising the step of introducing one or more factors to the containment area to enhance cell attachment and proliferation.

102. The method of claim 101, wherein the factor to enhance cell attachment and proliferation comprises serum proteins.

103. The method of claim 102, wherein the factor to enhance cell attachment and proliferation is fetal calf serum.

104. The method of claim 54, wherein the cell line is selected from the group consisting of human islets of Langerhans and insulin-producing endocrine cells.

105. The method of claim 54, wherein the cell line comprising progenitor cells.

106. The method of claim 104, wherein the progenitor cells are selected from the group consisting of pancreatic duct progenitor cells and islet-derived progenitor cells.

107. The method of claim 54, wherein the method of forming the fibrin matrix in the supporting container comprising the steps of:

providing a first solution comprising fibrinogen and factor XIII;

providing a second solution comprising thrombin and calcium;

mixing the first solution and the second solution thoroughly and rapidly to form a mixture;

introducing the mixture into the container and coating the mixture on a portion of the interior surface of at least one of the side walls of the supporting container; and

allowing the mixture to form a three-dimensional network of fibrin matrix on the inner surface of the side wall of the container.

108. The method of claim 107, wherein the fibrinogen is derived from mammalian plasma.

109. The method of claim 108, wherein the mammalian plasma is human plasma.

110. The method of claim 108, wherein the fibrinogen is a recombinant fibrinogen.

111. The method of claim 108, wherein the factor XIII is derived from mammalian plasma.

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112. The method of claim 111, wherein the mammalian plasma is human plasma.
113. The method of claim 106, wherein the factor XIII is a recombinant factor XIII.
114. The method of claim 106, wherein the thrombin is derived from mammalian plasma.
115. The method of claim 106, wherein the mammalian plasma is selected from the group consisting of bovine plasma and human plasma.
116. The method of claim 107, wherein the thrombin is a recombinant thrombin.
117. The method of claim 107, wherein the concentration of fibrinogen in the mixture in the first solution is from about 2.0 to about 20 mg/mL, the concentration of the factor XIII in the first solution is from about 10 to about 40 IU/mL, the concentration of the thrombin in the second solution is from about 2.5 IU/mL to about 50 IU/mL, and the concentration of the calcium in the second solution is from about 40 to about 100 umoles/mL. Approximately 0.5-1.0 mLs of the first solution is mixed with 0.5-1.0 mLs of the second solution to form a fibrin-forming mixture. The polymerization reaction takes place at room temperature in 1-5 minutes and is complete in about 5-15 minutes at 37°C. The fibrin matrix formed in this embodiment has a pore size of about 0.5 –5.0  $\mu\text{m}$  in diameter.
118. The method of claim 107, wherein the step of introducing the mixture to the containment area of the container is via an access port on the body of the container.
119. The method of claim 107 wherein the step of introducing the mixture to the containment area of the flexible gas permeable container is by spraying the mixture onto the interior surface of the side wall of the container.
120. The method of claim 54, wherein the method of forming the fibrin matrix in the supporting container comprises the steps of:
  - providing a dry fibrin matrix;
  - introducing the dry fibrin matrix into the supporting container; and
  - rehydrating the dry fibrin matrix in the supporting container.